GENETIC POLYMORPHISM IN CASEINS OF COWS' MILK. II. CONFIRMATION OF THE GENETIC CONTROL OF β-CASEIN VARIATION

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SUMMARY

Examination of the caseins from 1,349 individual cows of the five major dairy breeds in the United States showed that the A and B alleles of β-casein occur in Jerseys and Holsteins. The A, B, and C alleles occur in the Guernsey and Brown Swiss breeds. Only the A allele has been found in the Ayrshire breed. These results confirm the discovery of β-casein polymorphism by Aschaffenburg (1, 2) and support his conclusion that the variability has a genetic basis.

The rapidly expanding field of genetic polymorphism of proteins has been extended to include cow's casein, with the discovery of β-casein polymorphs by Aschaffenburg (1). By paper electrophoresis at pH 7.15 in 6.0 M urea, he demonstrated the existence of three forms of this protein which are called A, B, and C in order of decreasing mobility. The synthesis of these proteins is controlled by three allelic genes at a locus designated β-Cn. Aschaffenburg reported breed differences in the occurrence of the different forms of β-casein (2).

In our previous paper (5) describing the genetics of α_s-casein we observed that β-casein(s) could be typed simultaneously with α_s-casein, using starch-gel urea electrophoresis (SGE). This prompted us to study the genetics of β-casein polymorphism in American dairy cattle.

EXPERIMENTAL PROCEDURE

Selection of individual milk samples for study has been described (5). Initially, the genetic typing of both α_s- and β-caseins was performed, using SGE as described by Wake and Baldwin (7). With SGE, 7 mg of acid-precipitated casein was dissolved in 1 ml of 7.0 M, Tris-citrate buffer and 0.20 ml applied in the gel slot. In later studies, however, we found it expedient to dilute 1 ml of skimmilk with 2 ml of the urea buffer, which gave about a 1% casein solution. The slightly opaque solution (0.20 ml) was then applied in the gel slot.

More recently, we have used polyacrylamide gel electrophoresis (PAE) (6) to perform the genetic typing. An E-C Apparatus Company[2] vertical electrophoresis cell was used. The stock buffer is composed of 121 g reagent grade Tris (hydroxymethylaminomethane), 15.6 g Na_2-EDTA, and 9.2 g H_3BO_3 diluted to four liters with distilled water. One part of stock is diluted with two parts of water to give a pH 9.1-9.3 buffer solution. A stock gel solution is made by dissolving 70 g Cyanogum in about 400 ml of the buffer, adding 270 g urea, 1 ml β-dimethylamino propionitrile, and diluting to one liter with buffer. The resulting solution, which is 7.0% Cyanogum and 4.5 M urea, can be stored at room temperature for several weeks. To 150 ml of the gel stock is added 0.3 g ammonium persulfate, the solution poured, and the appropriate slot form inserted. The solution gels in about 20 min and is allowed to age 45 min before the run is commenced.

For typing purposes by PAE either 30 μl of a 1% solution of casein or 30 μl of skimmilk-buffer solution (described above for SGE) is layered in the gel slot. We have found (3), however, that whole skimmilk, with 8% sucrose to achieve the density required to layer the sample, may be added directly into the slot.

Current is applied to the gel as follows: 100 V (50-55 mA) for the first 15 min; 150V (60 mA) for the next 15 min, followed by slowly increasing the voltage to 250 but never exceeding 70 mA. The current drops with time, and after electrophoresis for 5 hr the final reading is 25-30 mA. The gel is removed, dyed for 3 min with Amido Black staining solution (7), excess dye washed out with 7% acetic acid,

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[2] It is not implied that the U. S. Department of Agriculture recommends products of companies mentioned to the possible exclusion of others in the same business.

and the gel decolorized for 20 min in aqueous 7% acetic acid, using an E-C Electrolytic Destainer.

RESULTS AND DISCUSSION

The electrophoretograms in Figure 1 (SGE) show separation of all six possible phenotypes of β-casein (A, AB, AC, B, BC, and C). Similarly, Figure 2 (PAE) shows the separation

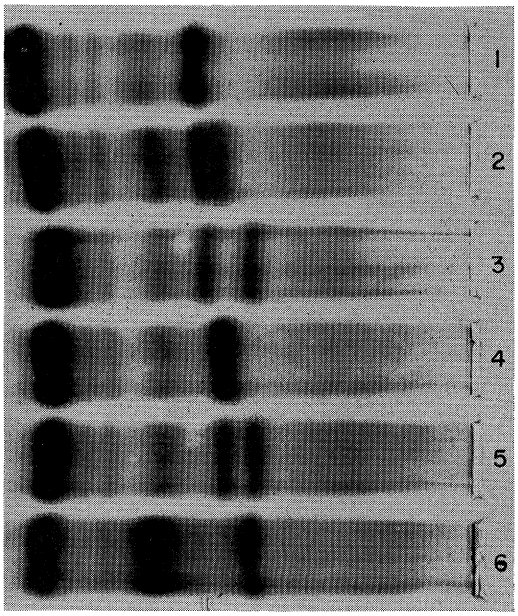

FIG. 1. Starch-gel electrophoresis patterns at pH 8.6, 7.0 M urea (7), of the six possible phenotypes of β-casein. Patterns 1-6 are β-A, AB, AC, B, BC, and C, respectively.

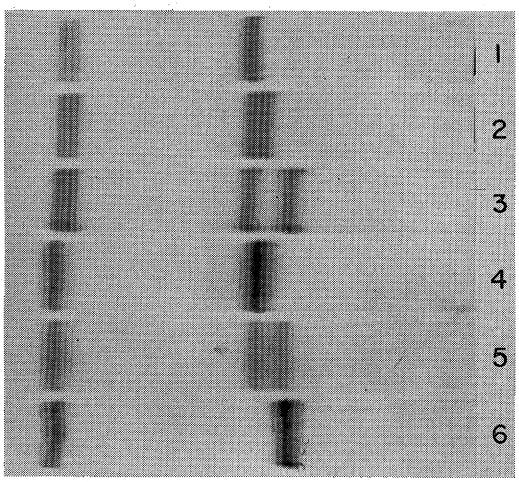

FIG. 2. Polyacrylamide-gel electrophoresis patterns, at pH 9.1-9.3, 4.5 M urea, of the six possible phenotypes of β-casein. Patterns 1-6 are β-A, AB, AC, B, BC, and C, respectively.

of the β-casein phenotypes. The figures show both zonal electrophoresis methods to be acceptable for the genetic typing of β-caseins. Paper electrophoresis (1) serves for typing of β-casein, but lacks the resolution of either PAE or SGE. Agar gel electrophoresis (AGE) at pH 6.0-6.5 (7) or at pH 8.6 in the discontinuous Tris-borate buffer system described by Wake and Baldwin (7) is satisfactory for β-casein typing. We believe, however, that PAE is superior to either SGE, paper, or AGE in overall resolution of components, ease of operation, and brevity of electrophoresis. Considering the number of samples required for a genetic survey, the direct use of skimmilk, instead of isolated whole caseins, is a distinct advantage. Furthermore, in a study of comparative biochemistry of milks from different species, where the quantity of milk is often limited, the use of skimmilk applied directly to the gel is expedient. For purposes of identification of zones by relative electrophoretic mobility on SGE (7), β-caseins A, B, and C are numbered 0.80, 0.76, and 0.70, respectively, as contrasted to values of 1.18, 1.10, and 1.07 for α_s-caseins A, B, and C, respectively. On PAE the numbers do not coincide with SGE and are found to be 0.65, 0.61, and 0.54 for β-caseins A, B, and C, respectively. α_s-Caseins A, B, and C are found to be 1.22, 1.13-1.14, and 1.10 by the PAE method.

Results of β-casein typing of 1,349 individual cows of the various breeds are shown in Table 1. The A and B forms of β-casein were found in four of the five breeds studied. The sole breed in which the B form was not found was Ayrshire. This observation is consistent with Aschaffenburg (2), who also observed that Shorthorn produced only the A form of β-casein. Of further interest is the observation that only the B form of α_s-casein has been reported in Ayrshire milks (5). In addition to A and B, the C form of β-casein was found in Brown Swiss and Guernsey cows, but in our survey we did not observe a homozygote β-casein C.

Aschaffenburg concluded that variation in β-casein was controlled by a series of three autosomal alleles (β-Cn^A, β-Cn^B, and β-Cn^C) with no dominance (2). The six possible phenotypes and their corresponding genotypes are A (A/A), B (B/B), C (C/C), AB (A/B), AC (A/C), and BC (B/C). Our results are in accord with this hypothesis. In 168 matings of the A/A × A/A type, all of the offspring were A/A. Sire types were deduced from progeny tests. Since the B and C forms of β-casein were relatively rare, it was not possible to make

TABLE 1

Occurrence of various β-casein types in different breeds

Breed	Total no. of cows tested	β-Casein types					
		A	B	C	AB	AC	BC
Ayrshire	98	98	0	0	0	0	0
Brown Swiss	202	124	4	0	68	5	1
Guernsey	400	385	0	0	3	12	0
Holstein	524	501	1	0	22	0	0
Jersey	66	28	9	0	29	0	0
Crossbreds:							
Brown Swiss × Holstein	29	26	0	0	2	1	0
Ayrshire × Holstein	21	21	0	0	0	0	0
Ayrshire × Brown Swiss	9	7	0	0	1	1	0
Totals	1,349	1,190	14	0	125	19	1
Per cent		88.0	1.0	0	9.3	1.4	0.1

segregation analyses for mating types other than A/A × A/A. However, among offspring from 13 matings of miscellaneous types results were as expected according to Aschaffenburg's hypothesis.

Gene frequencies were calculated and results are shown in Table 2. Since the samples were not collected in a completely random manner, these gene frequencies are presented only as preliminary information on the individuals we have studied. For comparison, we have calculated gene frequencies from the data presented by Aschaffenburg, which appear in Table 2. In both studies the β-Cn^A gene is by far the most common, and the agreement between the gene frequencies for the different breeds of cattle in the two studies is noteworthy. From these preliminary comparisons it appears that the breeds studied both in Great Britain and in the United States are similar with regard to the β-casein polymorphism. The apparent difference in gene frequency between the Channel Island breeds (Guernseys and Jerseys), pointed out by Aschaffenburg, is also seen in our data. In view of the low frequency of β-Cn^C in the Guernsey breed, however, it seems possible that this allele may yet be found in the Jersey breed, since the number of Guernseys studied to date is over three times the number of Jerseys. But, as Aschaffenburg points out, if the difference is a true one it may prove helpful in determining the ancestral backgrounds of the two breeds (2). Aschaffenburg suggests that studies of French breeds may be helpful in this respect. He also points out that the hemoglobin polymorphism would not be expected to help in differentiating between ancestors of the two breeds, since hemoglobins A and B are found in both breeds. The same could be said for the transferrin polymorphism, since Guernseys and Jerseys appear to have the same alleles in the system (Tf^A and Tf^D). (Refer to Ashton, G. C. Genetics of β-Globulin Polymorphism in British Cattle. Nature, 182: 370. 1958.) Of further interest in this regard is the finding by Bell (4) of a third β-lactoglobulin variant, C, in Jersey cattle. This finding has been confirmed by Aschaffenburg (3) and in our laboratory (unpublished results). If β-lactoglobulin C proves to be peculiar to the Jersey breed, then another milk protein polymorphism will be available for use in seeking out differences in the ancestral backgrounds of Guernseys and Jerseys.

The possibility exists that additional genetic variants will be observed in α_s- and β-casein and that polymorphism may be discovered in κ-casein. β-Lactoglobulin, for example, was considered to exist in only two forms, A and B. As pointed out above, however, recent SGE studies by Bell (4) indicate the existence of a

TABLE 2

β-Casein gene frequencies by breeds

Breed	Genes		
	β-Cn^A	β-Cn^B	β-Cn^C
Ayrshire	1.00(1.00) [a]	0 (0)	0 (0)
Brown Swiss	.79	.19	.02
Guernsey	.98(.89)	.004(.02)	.016(.09)
Holstein	.98(.95)	.02(.05)	0 (0)
Jersey	.64(.63)	.36(.37)	0 (0)
Crossbreds	.94	.05	.01
All breeds	.93(.85)	.06(.09)	.01(.03)

[a] Figures in parentheses are calculated from Aschaffenburg's data (see Reference 2).

third variant, named C. Furthermore, existence of Aschaffenburg's A_{slow} β-casein has been confirmed in our laboratory in milk from Holstein and Brown Swiss cows. The anomaly, present on paper electrophoresis, is eliminated when observed on PAE where two zones, typical of β-casein AB, are seen. This anomaly does not necessarily suggest additional polymorphism, since one of the two variants may be produced in lesser concentration, which may alter the electrophoretic pattern. However, this observation warrants further investigation.

The discovery of genetic variation in α_s- and β-caseins forces a re-evaluation of past work and opens up some new areas of research. Certainly, the physical-chemical data on these caseins must be re-evaluated, using preparations from homozygous cows. Such analyses are the object of current research. The existence of genetically determined forms of α_s- and β-casein (and possibly κ-casein) having different physical properties, as well as their quantity in milk, may be factors affecting heat stability and gel-forming characteristics of individual milks. The genetic variability among herds is often striking and we suspect that it may be important in explaining variation in physical properties of milk obtained from certain geographic locations.

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